Lucrări ştiinţifice **Zootehnie şi Biotehnologii**, vol. **41 (1)** (**2008**), Timişoara

THE HISTOLOGICAL AND BIOCHEMICAL CHANGES ON EXPERIMENTAL OSTEOARTHRITIS IN RABBIT KNEE

MODIFICĂRI HISTOLOGICE ŞI BIOCHIMICE ÎN OSTEOARTRITA EXPERIMENTALĂ LA IEPURE

HLUCHÝ S., ŠVÍK K. *, TOMAN R., MIKLÓŠOVÁ T., STANČÍKOVÁ M*., BÉLY M **, IŠTOK R.* , .FANO R*

Research Institute of Rheumatic Diseases, Pieštany, Slovak Republic
** Slovak University of Agriculture, Nitra, Slovak Republic*
*** National Institute of Rheumatology and Physiotherapy, Budapest, Hungary*

Osteoarthrosis (OA) is a common joint disease, characterized by degenerative changes in articular cartilage and reactive proliferation of bone and cartilage around the joint. The evaluation of the histological and biochemical changes were investigated on the articular cartilage of knee joint during development of osteoarthritis in rabbits following anterior cruciate ligament transsection (ACLT). A total of 12 New Zealand White (NZW) male rabbits with closed epiphyses underwent ACLT of knees and healthy control knees arthrotomy. The animals were killed at 9 weeks after surgery. The parameters tested were gross morphology, histology as well as urinary pyridinoline (Pyr) and creatinine. Morphological changes in osteoarthritic femoral condyles were seen on both, medial and lateral region, but markedly on medial condyle of the ACLT knee. The articular cartilage was characterised by a rough and hypertrophic appearance with severe erosions. The gross morphological examination of healthy control revealed no changes. Histological evidence for cartilage degeneration was observed in the ACLT knees. In OA femoral condyles the articular cartilage showed degenerative changes, including: rough surface, loss of superficial layer, erosion, fissures, irregular arrangement and form of chondrocytes. Biochemical determinations showed significantly higher concentrations of urinary Pyr in the OA rabbits compared to healthy rabbits during the whole period of the study (9 weeks), indicating a higher collagen degradation of cartilage and subchondral bone in the osteoarthritic animals.

Key words: rabbit, osteoarthritis, model, gross morphology, histopathology, pyridinoline

Introduction

Osteoarthritis (OA) is one of the most common causes of physical disability in the civilized countries. It is characterized by a loss of cartilage, bone

remodelling and a synovial reaction resulting in a narrowing of the joint space, subchondral sclerosis and formation of marginal osteophytes (Bagge, et al., 1991). Therefore, animal models as those used in this study, are frequently employed for a more exact evaluation of pathophysiology or the investigation of the efficacy of new drugs (Moskowitz, et al,. 1992). Experimentally induced OA in animals has been employed for many years using a variety of techniques including immobilisation of the joint, surgical alteration and destabilisation of the joint architecture, as well as intra-articular injection of destructive agents (papain, chondroitinase) (Evans et al., 1960).

The aim of this study was therefore the evaluation of on the articular cartilage of knee joint during development of OA in rabbits following anterior cruciate ligament (ACL) transsection using the model described by Yoshioka et al. (1996).

The parameters tested were gross morphology, histology as well as urinary pyridinoline (as a marker of collagen degradation). Concerning the latter, Thompson et al. (1992) and the others (Robins et al., 1986) demonstrated that urinary levels of pyridinoline significantly correlate with cartilage damage and with the X-ray-based rating of osteoarthritic joints.

Materials and Methods

Experimental animals

A total of 12 New Zealand White (NZW) male rabbits weighing 2.53 ± 0.09 kg each were used in this study. The rabbits were randomly divided into two groups of 6 animals after anterior cruciate ligament transsection (ACLT) of right knees and simple arthrotomy of their contra-lateral left knees. Group 1 – healthy control (HC) and group 2 - osteoarthritic rabbits (OA) were sacrifice at 9 weeks after surgery.

Surgical procedure

All rabbits were anaesthetised by intramuscular injection of ketamine (100 mg/kg) and xylazine (8mg/kg). Following the anaesthetizes, both knees were shaved and disinfected with betadine solution. A medial parapatellar incision was made on the skin followed by arthrotomy. The patella was dislocated laterally and the knee placed in full flexion.

The ACL was then transsected. After transsection the joint was irrigated with sterile saline and closed. The capsule and the synovium were closed with a running suture of 2-0 silon. The skin was closed in the same manner with additional interrupted sutures of 3-0 silon. The sham controls received the same treatment except for the transsection of the ACL.

Post-operatively the animals were permitted cage (60x50x40 cm) activity. The animals were closely monitored for infections and other complications. The average weight of the rabbits at surgery was 2.53 ± 0.09 kg, and at death 3.37 ± 0.13 kg.

Histological analysis

Both medial and lateral femoral condyles of the right and left knees were used for histological preparation and assessment. The tissues were fixed in 10% formaldehyde solution, decalcificated at room temperature in EDTA at pH 7.6. After decalcification, the femoral condyles were cut along the sagittal plane and both medial and lateral condyles were embedded in paraffin. Five micron sections were cut with a Reichter - Jung microtome and stained with haematoxyline, eosin and safranin O.

The conditions of articular cartilage were characterized histologically based on regressive changes of cartilage such as : loss of superficial layer, superficial erosion, fibrillation and fissures of cartilage, loss of proteoglycan, disorganisation of chondrocytes, loss of chondrocytes, exposure of subchondral bone, cluster formation obtained in table 1 according to Kikuchi et al. (1996).

Table 1.

Method of histopathological evaluation of cartilage degeneration

	+1	+2	+3	+4
Loss of superficial layer	<Slight	Moderate	Focally severe	Extensively severe
Erosion of cartilage	<Detectable	Moderate	Focally severe	Extensively severe
Fibrillation and/or fissures	<Noticeable (<1 very small)	Moderate (1 small)	Marked (2 small or 1 medium)	Extensive (3 small, 2 medium or 1 large)
Loss of proteoglycan	<Paler stain than control	Moderate loss of safraninophilia	Marked loss of safraninophilia	Total loss of safraninophilia
Disorganization of chondrocytes	Noticeable	Moderate with some loss of columns	Marked loss of columns	No recognizable organization
Loss of chondrocytes	<Noticeable decrease in cells	Moderate decrease cells	Marked decrease in cells	Very extensive decrease in cells
Exposure of subchondral bone	< Focal exposure of bone	Moderate exposure of bone	Fairly extensive exposure of bone	Very extensive exposure of bone
Cluster formation	<3-4 small, or 1-2 medium	5-6 small, 3-4 medium or 1-2 large	7 or more medium or 5-6 large	7 or more small, 5-6 medium or 3-4 large

Biochemical determination

24-hour urine samples were collected from the rabbits in the metabolic cage during the 9 weeks and stored at -60^{o} C until analysis. Pyridinoline (Pyr) was determined in the pooled urinary samples from three rabbits. The urinary samples were supplemented by 6 samples of healthy rabbits without surgery (normal values). The samples were hydrolysed at 110^{o} C in 6 M HCl for 18 hours. Urinary Pyr corrected for creatine was then measured by high performance ion-exchange chromatography as described elsewhere (6) with some slight modifications. Small solid phase extraction (SPE) columns filled with microparticulate cellulose (approx. 1 ml) were conditioned with 12 ml of mobile phase (n-butanol:acetic acid:water = 4:1:1). Following the conditioning, 0.5 ml of hydrolysate was mixed with 0.5 ml of glacial acetic acid and 2 ml of n-butanol and applied to the SPE columns. After washing with the mobile phase (3 x 4 ml), pyridinoline crosslinks were eluted from the columns by 1.8 ml of mobile phase used for the subsequent high performance liquid chromatography (HPLC) analysis on HEMA BIO 1 000 SB 250 x 4 mm columns (Tessek, Czech Republic). The mobile phase was prepared by mixing 0.45 M sodium sulphate and 0.3 M acetate buffer, pH 3.0 at a ratio of 9 : 22. Analysis was performed isocratically at a flow rate of 0.8 ml/min. A fluorescence detector was used for the analyses (excitation at 295 nm and detection at 400 nm).

Statistical analyses of histological results were carried out using statistical method, one-way analysis of variance (ANOVA) for paired data sets with a level of significance at $p<0.05$.

Results and Discussions

Histological evaluation

Histological evidence for cartilage degeneration was observed in the ACLT knees of osteoarthritic rabbits. The surface of articular cartilage of healthy controls was smooth without erosions, fibrillation and fissures with a normal histological appearance. The matrix was intact and the arrangement of chondrocytes was regular. In OA controls the articular cartilage showed degenerative changes, including: rough surface, loss of superficial layer, erosion, fibrillation and/or fissures, irregular arrangement of chondrocytes. Necrotic chondrocytes (without nuclear staining) were rarely seen, and were sporadically mixed with hypertrophic (enlarged) and hyperchromic (hyperfunctional) chondrocytes.

Histopathological score

The mean global histopathological score in healthy group was significantly lower in medial condyles (4.00 ± 0.45, $p < 0.001$) in comparison with OA group (23.33 ± 0.54) (Table 2.). Similar results were observed in lateral condyles. The items of the histopathological score in the healthy rabbits were usually significally lower than in OA controls.

Table 2.

Histopathological scores in healthy and OA groups of rabbits.

	HC	OA
Femur - medial condyle		
Global score	4.00 ± 0.45	23.33 ± 0.54
Loss of superficial layer	0.83 ± 0.41	3.17 ± 0.41
Erosion of cartilage	0.00 ± 0.00	3.00 ± 0.89
Fibrillation and/or fissures	0.17 ± 0.41	3.17 ± 0.41
Loss of proteoglycan	1.17 ± 0.41	3.33 ± 0.82
Disorganization of chondrocytes	1.00 ± 0.00	3.50 ± 0.55
Loss of chondrocytes	0.50 ± 0.55	3.00 ± 0.63
Exposure of subchondral bone	0.17 ± 0.41	2.17 ± 0.41
Cluster formation	0.17 ± 0.41	2.00 ± 0.00
Femur - lateral condyle		
Global score	2.33 ± 0.25	18.83 ± 0.60
Loss of superficial layer	0.67 ± 0.52	2.17 ± 0.41
Erosion of cartilage	0.17 ± 0.41	2.17 ± 0.75
Fibrillation and/or fissures	0.17 ± 0.41	2.17 ± 0.75
Loss of proteoglycan	0.67 ± 0.52	3.17 ± 0.75
Disorganization of chondrocytes	0.33 ± 0.52	3.17 ± 0.75
Loss of chondrocytes	0.17 ± 0.41	2.50 ± 0.55
Exposure of subchondral bone	0.17 ± 0.41	2.17 ± 0.75
Cluster formation	0.00 ± 0.00	1.33 ± 0.52

Significantly different from OA control, $p < 0.001$in all parameters

Biochemical determinations

Concentrations of urinary Pyr were significantly higher in the OA group compared to healthy rabbits during the whole period of the study (9 weeks), indicating a higher collagen degradation of cartilage and subchondral bone in the osteoarthritic animals.

Joint destabilisation secondary to a complete transection of the ACL is known to cause a breakdown of articular cartilage with the resulting loss of joint function. Nine weeks after surgery, inspection of the joints revealed an extensive damage in the ACLT knees of all osteoarthritic animals. All tested parameters indicated severe osteoarthritis: roughness of the cartilage surface, the thickness of the cartilage, number and distribution of chondrocytes as well as the morphology of these cells. Morphological grading of the femoral condyles and the histological scores in ACLT knees revealed a significantly lower extent and severity of cartilage damage in healthy rabbits compared to the OA control animals. Urinary pyridinoline levels in the ACL transected animals were higher in control OA group supporting on a biochemical basis the view of cartilage protection by healthy group.

In conclusion, the results of our morphological and histological experiments as well as the biochemical findings demonstrate a significant and substantial

increase in severity of the damage caused in condyle cartilage and in chondrocytes as the features of the development of joint damage in the ACLT rabbit model of osteoarthritis.

Acknowledgement

We would like to thank for financial support to the Scientific Grant Agency (VEGA) of the Ministry of Education of Slovak Republic (Project No. 2 / 6023 / 06).

Bibliography

1. **Bagge, E., Bjelle, A., Eden, S., Svanborg, A.** (1991)- *Osteoarthritis in the elderly*. Ann Rheum Dis, 50, p. 535-9.
2.**Evans,E.B., Eggers, G.W.N., Butler, J.K., Blumel, J.**(1960)-*Experimental immobilization and remodelization of rat knee joints.* J. Bone Joint Surg, 42A, p. 737-58.
4. **Hulth, A., Lindberg, L., Telhag, H.** (1970) - *Experimental osteoarthritis in rabbits: preliminary report.* Acta Orthop Scand, 41, p. 522 - 30.
5. **Kikuchi,T., Yamada,H., Shimmei,M**.: (1996) - *Effect of high molecular weight hyaluronan on cartilage degeneration in a rabbit model of osteoarthritis.*Osteoarthritis and Cartilage, 4, p. 99 - 110.
6. **Lichý, A., Macek, J., Adam, M.(** 1991) - *Determination of hydroxylysilpyridinoline in tissues and urine by high-performance liquid chromatography*. J. Chrom 563, , p. 153 - 157.
7. **Moskowitz, R.W.** (1992) - *Experimental model of osteoarthritis.* In **Moskowitz RW, Howell DS, Goldberg VM, Mankin HJ, Eds**. *Osteoarthritis: diagnosis and Medical/Surgical Managment*, 2nd ed. Philadelphia, PA:W.B. Saunders, p. 213 - 32.
8. **Murray, D.G.** (1964) *Experimentally induced arthritis using intra-articular papain.* Arthritis Rheum 7, p. 211-9.
9. **Robins, S.P., Stewart, P., Astbury, C., Bird, H.A.** (1986) - *Measurement of the cross-linking compound, pyridinoline, in urine as an index of collagen degradation in joint disease*. Ann Rheum Dis, 45, p. 969-973.
10. **Sinigaglia, L., Varenna, M., Binelli, L., Bartucci, F., Arrigoni, M., Ferrara, R.,Abbiati, G.** (1995) - *Urinary and synovial pyridinium crosslink concentrations in patients with rheumatoid arthritis.* Ann Rheum Dis, 54, p. 144 - 147.
11. **Thompson, P.W., Spector, T.D., James, I.T., Henderson, E., Hart, D.J.** (1992) - *Urinary collagen crosslinks reflect the radiographic severity of knee osteoarthritis.* Br. J. Rheumatol., 31, p. 759 - 761.
13.**Yoshioka, M., Coutts, R.D., Amiel, D., Hacker, S.A.** (1996) - *Characterization of a model of osteoarthritis in the rabbit knee.* Osteoarthritis and Cartilage, 4, p. 87-98.